R^4 is Gln-Ser.

C2 cont

11. (Once amended) The method of claim 1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly (SEQ ID NO:8);

z is one; and

R^4 is Gln-Ser.

C3

13. (Once amended) The method of claim 1, wherein x, y, w, and z are all zero.

REMARKS

With this amendment, claims 1-18 are pending in the present application and are currently under examination. Claims 19-44 are currently withdrawn from consideration as being drawn to non-elected inventions and have been canceled. Claims 2, 3, and 14 are canceled. For convenience, the Examiner's rejections are addressed in the order presented in the June 29, 2001 Office Action. Appendix A provides the version with markings to show changes made. Also for the Examiner's convenience, Appendix B is included listing all pending and amended claims.

1. Status of the Claims

Claim 1 has been amended to delete elements d, e, and f, thereby complying with the sequence requirement of 37 CFR 1.821-1.825.

Claims 2, 3, and 14 are canceled.

Claims 4-11 and 13 formerly depended on claim 3. These claims have been amended and are now dependent on claim 1. These amendments add no new matter.

2. Sequence Requirements

The Examiner alleged that the application failed to comply with the requirements of 37 CFR 1.821 through 1.825. Specifically, the Examiner asserted that the newly amended sequences in claim 1, elements d-f, lacked a reference to an identifiable SEQ ID NO as required by the sequence rules.

Applicants respectfully point out that claim 1 has been amended to remove elements d-f. Therefore, the Application now fully complies with the requirements of the sequence rules, 37 CFR 1.821-1.825.

3. Rejection under U.S.C. §112, first paragraph: written description

Claims 1, 2, and 12 were rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification as originally filed. In the Office Action the Examiner observed that the purpose of the written description requirement is to convey to one skilled in the relevant art that the inventors had possession of the claimed invention as of the filing date. The Examiner went on to state that "[T]he skilled artisan cannot envision the detailed chemical structure of the encompassed nucleic and amino acids...The specific nucleic and amino acids are required." Office action at page 4.

To the extent that the rejection applies to the claims as amended, Applicants respectfully traverse. The application fully complies with the requirements for written description of a chemical genus as set forth in *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997). Quoting *Fiers v. Revel* the court stated that an adequate written description for a chemical genus "requires a precise definition, such as by structure, formula, chemical name, or physical properties." (See Lilly at 1405, quoting *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993)). As described by the Federal Circuit in *Lilly*, "[a] description of a genus of cDNAs may be achieved by "recitation of a representative number of [species]. . . **or** a recitation of structural features common to the members of the genus . . ." *Lilly*, 43 USPQ2d at 1406 (emphasis added).

The present invention relates to methods of using ADNF I and ADNF III polypeptides to reduce a condition associated with fetal alcohol syndrome in a subject who is exposed to alcohol *in utero*. Various embodiments of the ADNF I polypeptide are described in the specification; all include the conserved core active site SALLRSIPA, referred to as "SAL." Various embodiments of the ADNF III polypeptide are described in the specification; all include the conserved core active site NAPVSIPQ, referred to as "NAP." SAL and NAP are the smallest peptides that exhibit the full efficacy of native ADNF I and ADNF III, respectively. Specification at page 3, lines 11-20.

The genus of ADNF I polypeptides is specifically described in the present application by a chemical formula, which provides the SAL core sequence plus up to 40 additional amino acid residues at either or both the C- and N-terminus. Similarly, the genus of ADNF III polypeptides is specifically described in the present application by a chemical formula which provides the NAP core sequence plus up to 40 additional amino acid residues at either or both the C- and N-terminus. More particularly, the formula for the claimed ADNF I and ADNF III polypeptides reads as follows:

ADNF I: $(R^1)_x$-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala-$(R^2)_y$ (SEQ ID NO:3, comprising the SAL core sequence);

ADNF III: $(R^3)_w$-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-$(R^4)_z$ (SEQ ID NO:4, comprising the NAP core sequence);

wherein R^1, R^2, R^3, and R^4 are independently selected and are an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected; and x, y, w, and z are independently selected and are equal to zero or one.

Specification at page 3, line 30 to page 4, line 6. Thus, every member of the ADNF I genus contains the SAL core sequence and every member of the ADNF III genus contains the NAP core sequence.

As required by the standard set forth in *University of California v. Lilly*, this formula describes the structural features common to all of the members of the ADNF I polypeptide genus and the structural features common to all of the members of the ADNF III polypeptide genus. This sequence represents the structural feature (e.g. either SAL or NAP) required for membership in the ADNF I polypeptide genus or for

membership in the ADNF III polypeptide genus. Furthermore, the specification provides numerous specific embodiments of both genera, (*see e.g.*, the preferred peptides described in the specification on page 18, lines 19-30).

The conserved core active site domains "clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed." *Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 111, 1116 (Fed. Cir. 1991). The specification thus appropriately describes both the claimed ADNF I protein genus and the ADNF III protein genus using structural/physical features, as required by the court in *University of California v. Eli Lilly*. As such, Applicants respectfully request that the Examiner withdraw the rejection for failure to meet the written description requirement.

4. Rejection under U.S.C. §112, first paragraph: enablement

Claims 1-18 were rejected under 35 U.S.C. §112, first paragraph as allegedly lacking enablement. The standard for enablement cited by the Examiner is that the specification must enable one skilled in the art to be able to make and use the invention commensurate in scope with the claims.

While Applicants believe the specification fully enables the claims as written, in order to expedite prosecution and permit the earliest possible date of allowance, Applicants have canceled claim 14. To the extent that the rejection applies to the amended claims, Applicants respectfully traverse.

The Examiner raised four objections based on enablement. First, the Examiner appeared concerned that Applicants had not provided a working example for treatment with ADNF polypeptides after ethanol administration. Applicants have included a declaration from Dr. Brenneman, an inventor, which provides support that ADNF polypeptides given after ethanol administration are able to reduce a condition associated with fetal alcohol syndrome. The Examiner cautioned against using a rodent model to study fetal alcohol syndrome. However, the reference cited by the Examiner is not relevant to the claimed methods. Furthermore, as described in the declaration of Dr. Brenneman, Applicants are using an art-accepted model to study fetal alcohol syndrome.

The Examiner stated that the claims failed to specify a dosage for use of ADNF polypeptides in the claimed methods. The declaration of Dr. Brenneman provides support that at least one disclosed embodiment provides an effective range of ADNF polypeptide. Furthermore, Applicants assert the specification provides guidance for one of skill in the art to determine an appropriate dosage range for ADNF polypeptides. With regard to claimed variants of the ADNF polypeptides, the Examiner appeared to focus on the alleged possibility of inoperative embodiments. Given the guidance provided in the specification and the knowledge of those of skill in the art, Applicants assert the Examiner's focus on inoperative embodiments is improper.

A. Timing of treatment with ADNF polypeptides.

The Examiner objected to a model of fetal alcohol syndrome treatment where subjects were treated with the ADNF polypeptides NAP and SAL before exposure to alcohol. Office action at page 6. The Examiner stated that the protocol failed to "support a reduction in a condition associated with developed fetal alcohol syndrome." *Id.*

Applicants have included a declaration under 37 C.F.R. 1.132 from Douglas E. Brenneman, Ph.D. and a published manuscript authored by Dr. Brenneman and his co-inventors (Exhibit 3). Both documents demonstrate that treatment with ADNF I and ADNF III polypeptides after alcohol administration reduces conditions associated with fetal alcohol syndrome.

In brief, the experiments were done by administering alcohol to pregnant mice and treating with SAL, the ADNF I core active site polypeptide (referred to as ADNF-9 in the published manuscript and the declaration), and NAP, the ADNF III core active site polypeptide. The ADNF polypeptides were given both before and after alcohol administration. Animals that were given alcohol alone had litters with severe impairments compared to litters from control animals that did not receive alcohol. The fetal survival rate of mice treated with NAP or with a combination of NAP and SAL polypeptides thirty minutes before alcohol administration was significantly higher than

that of fetuses from mice given alcohol alone. In addition, the experiments described in the declaration show that treatment with NAP, alone or in combination with SAL, one hour after alcohol administration also had a fetal survival rate significantly higher than that of fetuses from mice given alcohol alone.

Treatment with NAP and SAL also protected fetuses from growth abnormalities resulting from maternal alcohol intake during pregnancy. Treatment with SAL and NAP polypeptides one or three hours after alcohol administration resulted in brain weights significantly greater than those of fetuses from mice treated with alcohol alone. This protective effect mimics that of treatment with SAL and NAP before alcohol administration.

One of skill in the art would have been able to make and use the invention based on the disclosure in the specification. Demonstration that ADNF polypeptides are effective one or three hours after alcohol administration would not have required undue experimentation on the part of one skilled in the art. Applicants thus respectfully request that the rejection of the claims for lack of an enabling disclosure be withdrawn.

B. Use of rodent models for fetal alcohol syndrome.

The Examiner cited an abstract by Hannigan *et al.* to caution against using basic research models to assess potential treatments for neurobehavioral effects of prenatal alcohol exposure. Office action at page 7. According to the Examiner, the reference stated that application of findings in rats may not be straightforward in children. *Id.*

i. The cited reference does not does not apply to the research model used in the current invention.

In both the abstract and the full text of the article, Hannigan *et al.* had the goal of assessing the use of **environmental** enrichment for children with Fetal Alcohol Syndrome (FAS). Hannigan *et al.* at page 104. The reference assessed the effect of post-birth 'enriching' learning environments on rodent subjects that had been given alcohol in

utero. Thus, the reference describes models for modulating learning and behavior of children with fetal alcohol syndrome by providing specified environments.

In contrast, Applicants provide a preventive therapeutic compound. Applicants do not attempt to manipulate the environment around the experimental subjects. Applicants assert ingestion of the compound will prevent the need for such specified learning environments.

In addition, Hannigan *et al.* does not suggest that all basic research models for treating the effects of prenatal alcohol exposure are unpredictable. On the contrary, the reference appreciates the usefulness of the rodent model for those of skill in the art, stating:

> Animal models, and rodent models in particular, have proven effective in characterizing the phenomenology, pathology, and risk factors associated with maternal alcohol consumption during pregnancy. The biological and behavioral outcomes of prenatal exposure in these animals can be remarkably consonant with the clinical picture in humans.
> *Id.* at page 105.

The present invention does not attempt to change the environments of subjects exposed to alcohol *in utero*. Rather, Applicants disclosed an ingested substance that reduces conditions associated with fetal alcohol syndrome when taken internally by the afflicted subject. As the present claims are not directed toward providing an 'enriching' learning environment useful for treating the effects of prenatal alcohol exposure, the reference does not demonstrate the specification lacks enablement and is not relevant to the claims. Thus, Applicants respectfully request that the rejection on this basis of non-enablement be withdrawn.

ii. The research model used in the current invention is an art-accepted model of the effects of fetal alcohol syndrome.

As stated by Dr. Brenneman in his declaration, the model used in the experiments is an art-accepted model for fetal alcohol syndrome. The model uses intraperitoneal injection which allows accurate and reproducible administration of

alcohol. (Webster *et al. Neurobehav. Toxicol.* 2:227-234 (1980) (Exhibit 2)) This administration method was also shown to produce a high blood alcohol concentration, providing a stringent test of the efficacy of the ADNF polypeptides. A high alcohol dose was selected, again providing the most severe test of the efficacy of ADNF polypeptides. Gestational day 8 was chosen for administration of alcohol, again because it provided the most severe test of the biological activity of the ADNF polypeptides.

According to the MPEP, "if the art is such that a particular model is recognized as correlating to a specific condition, then it should be accepted as correlating unless the examiner has evidence that the model does not correlate." MPEP § 2164.02. Applicants have demonstrated that the model is art-accepted model and moreover, is one of the most stringent tests of whether a compound is effective in reducing a condition associated with fetal alcohol syndrome. Without evidence that the mouse model does not correlate to a specific condition, the rejection of the claims based on use of the model should be withdrawn.

iii. Exact Correlation between In Vivo Models and Human Conditions Not Required

At page 9 of the Office Action, the Examiner acknowledges that Applicants have provided sufficient guidance for the embodiments exemplified in figures 1, 2a, 2b, and 3, e.g. reduction of conditions associated with fetal alcohol syndrome in a mouse. However, the Examiner goes on to state that guidance is not provided for other methods to ameliorate fetal alcohol syndrome conditions, and that the specification is allegedly not enabling in that respect. The Examiner appears to be concerned that the animal models may not correlate exactly with results in human subjects.

Applicants respectfully traverse and submit that 35 U.S.C. § 112, first paragraph, does not require such standards. As MPEP § 2164.02 states, "[a] rigorous or an invariable exact correlation is not required" between a particular model and a specific condition. Moreover, as the Court of Appeals for the Federal Court stated, "[t]itle 35 does not demand that such human testing occur within the confines of Patent and

Trademark Office, (PTO) proceedings." *In re Brana*, 51 F.3d 1560 (Fed. Cir. 1995). In addition, "[t]here is no decisional law that requires an applicant to provide data from human clinical trials to establish utility for an invention related to treatment of human disorders." *See* MPEP § 2164.02.

As described above, Webster *et al.* provide an art-accepted and rigorous model to test the efficacy of ADNF polypeptides in reducing conditions associated with fetal alcohol syndrome. In addition, the specification, coupled with the information known in the art at the time of the invention, provides ample guidance for one of skill to test the biological activity of ADNF polypeptides. Applicants respectfully request that this rejection for lack of enablement be withdrawn.

C. Dosage of ADNF polypeptides.

The Examiner also objected to the claim limitation "in an amount sufficient to reduce the condition associated with fetal alcohol syndrome." The Examiner went on to state that the claims "failed to specify any amount in correlation to any specified condition associated with fetal alcohol syndrome." Office action at page 7.

The Office Action is in error. The specification clearly teaches one of skill in the art the appropriate dosage of the ADNF peptide to use. The specification discloses that "an amount sufficient" is that amount of ADNF peptide that reduces fetal alcohol syndrome. The specification then gives examples of how to measure a reduction in fetal alcohol syndrome after ADNF administration: 1) by measuring a reduction of fetal deaths after alcohol intake, 2) by measuring a reduction of diminished fetal body or brain weights after alcohol intake, or 3) by preventing reduction of VIP mRNA levels after alcohol intake. Specification at page 8, lines 24-32.

The specification also teaches one embodiment of a dosage range of ADNF that reduces fetal alcohol syndrome. The disclosed range is 1 μg-50 μg per mouse and the dose is based on the body weight of the mouse. One of skill in the art would be able to extrapolate that dosage to a human, based on the body weight of the human. Specification at page 19, lines 19-22.

The specification also includes examples demonstrating specific dosages of ADNF peptides that lead to a reduction of specific conditions associated with fetal alcohol syndrome. Treatment of mice with ADNF peptides led to a decreased percentage of fetal death after alcohol administration. Specification at page 35, lines 10-23. Treatment of mice with ADNF peptides prevented fetal brain and body weight reductions after alcohol administration. Specification at page 35, lines 24-27. Treatment of mice with ADNF peptides prevented reduction in VIP mRNA levels after alcohol administration. Specification at page 36, lines 10-16. In the examples, the total dosage of ADNF peptides was 40 μg, within the disclosed range of 1μg-50μg of ADNF peptide per mouse.

Applicants refer the Examiner to the declaration under 37 C.F.R. 1.132 from Douglas E. Brenneman, Ph.D. and the published manuscript authored by Dr. Brenneman and his co-inventors. At paragraph 9 of the declaration, Dr. Brenneman describes how pregnant mice were given twenty micrograms of [^{3}H]NAP, the ADNF III conserved active site domain. When fetal tissues were analyzed later, intact [^{3}H]NAP was recovered from the fetus in a concentration within the therapeutic range of the ADNF III polypeptides. This demonstrates that the dosage range of ADNF polypeptides disclosed in the embodiment includes an appropriate dosage of the peptide. Thus, one of skill in the art could readily determine an appropriate dosage range for human subjects.

Thus, Applicants have disclosed amounts of ADNF to use and have correlated that amount with a reduction in specific conditions associated with fetal alcohol syndrome. Furthermore, using the assays of the specification, one of skill in the art could readily determine an appropriate dosage without undue experimentation. Applicants therefore respectfully request that the Examiner withdraw this rejection for failure to enable one of skill in the art to use the invention.

D. Prediction of protein function based on structure.

The Examiner cited Skolnick *et al.* to state that for "divergent peptide structures, the skilled artisan would be required to perform further undue experimentation

to discover those ADNF peptides which possess the properties of alleviating any condition associated with fetal alcohol syndrome."

The Examiner appears to have focused improperly on inoperative embodiments, leading to the conclusion that undue experimentation would be required to identify biologically active peptides that carry out the methods of the claimed invention. However, the proper test of enablement is "whether one skilled in the art could make or use the claimed invention from the disclosure in the patent coupled with information known in the art without undue experimentation" (*see, e.g.*, MPEP §2164.01). In the present application, one of skill would know how to avoid inoperative embodiments and make biologically active polypeptides, without undue experimentation (*see, In re Cook and Merigold*, 169 USPQ 299, 301 (C.C.P.A. 1971)). Moreover, the present application provides guidance in the form of assays and working examples for identification of biologically active ADNF polypeptides.

i. One of skill in the art would know how to avoid inoperative embodiments.

Claims reading on inoperative embodiments are enabled if the skilled artisan understands how to avoid inoperative embodiments. As described by the court in *In re Cook and Merigold*, 169 USPQ 302:

> Many patented claims read on vast numbers of inoperative embodiments in the trivial sense that they can and do omit 'factors which must be presumed to be within the level of ordinary skill in the art'....There is nothing wrong with this so long as it would be obvious to one of ordinary skill in the relevant art how to include those factors in such a manner as to make the embodiment operative rather than inoperative.

See, In re Cook and Merigold, 169 USPQ at 302 (quoting in part *In re Skrivan*, 166 USPQ 85, 88 (C.C.P.A. 1970)).

Because the core active sites of ADNF I and ADNF III are well characterized and well known in the art, application of predictive structural analysis to determine the active site, as suggested by Skolnick *et al.*, is not required. Applicants

claim methods of using proteins with identical amino acid sequences at a core active site. The specification discloses that additional amino acids can be added to both the N-terminus and the C-terminus of the active site domains without loss of biological function because the full-length ADNF I and ADNF III polypeptides have extraordinary biological activity. Specification at page 18, lines 30-33. In addition, for both ADNF I and ADNF III, the specification discloses preferred peptides with additional amino acids at page 18, lines 19-30.

The Examiner appears concerned that if one of skill in the art choose to add amino acids to an ADNF core active site, the skilled artisan would likely choose to make an inoperative embodiment. The Examiner's concern is misplaced for the following reasons. 1) The preferred and active peptides listed in the specification at page 18, lines 19-30, give guidance as to which amino acids are suitable for addition to the core active site peptides of ADNF I and ADNF III. 2) The properties of amino acids are well known by those of skill in the art. Amino acids are characterized by their hydrophobicity, charge, and bulk of side chains, for example. Knowing the properties of particular amino acids, the skilled artisan could easily choose appropriate amino acids to add to the core active sites and could avoid adding amino acids that would be detrimental to the structure or function of the polypeptide. In addition, those of skill in the art are aware of methods, like alanine scanning, where amino acid sequences are manipulated with minimal disruption of protein structure or function.

ii. The specification teaches routine assays for identification of biologically active ADNF polypeptides.

Factors such as the amount of guidance presented in the specification and the presence of working examples must be considered to determine whether undue experimentation is required to practice the claimed invention (*see, Ex Parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int. 1985) and *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988)). As described in *Wands*, " a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a

reasonable amount of guidance with respect to the direction in which the experimentation should proceed" (*see*, *Wands*, USPQ2d at 1404, quoting *In re Jackson*, 217 USPQ 804 (Bd. Pat. App. & Int. 1982)).

As described above, the specification teaches routine methods for making biologically active ADNF I and ADNF III polypeptides. Furthermore, the specification also provides standard assays and working examples for identifying ADNF polypeptides that will reduce a condition associated with fetal alcohol syndrome. Identification of biologically active ADNF polypeptide is, therefore, well within the means of one of skill of the art, without undue experimentation. Assays for activity of ADNF I and ADNF III polypeptides can be found at page 19, lines 5-29. The assays use an art-accepted model for research on the effects of fetal alcohol syndrome. A working example of reduction in a condition associated with fetal alcohol syndrome by treatment with ADNF I or ADNF III is provided at page 34, line 22 through page 37, line 10.

Applicants have demonstrated that the claimed invention is enabled with respect to the timing of treatment with ADNF polypeptides, the use of a rodent model for fetal alcohol syndrome, the appropriate dosage of the ADNF polypeptide, and prediction of function based on the presence of a core active site domain. Applicants thus respectfully request that the rejection on the basis of nonenablement be withdrawn.

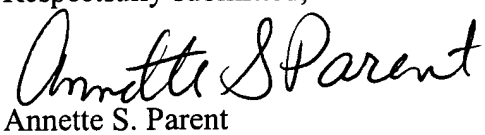
5. Rejection under U.S.C. §112, second paragraph

Claims 1-18 were rejected under U.S.C. §112, second paragraph as being indefinite. Applicants continue to assert the claims as filed point out and distinctly claim the subject matter of the invention. However, in order to expedite prosecution and to receive the earliest possible date of allowance, Applicants have amended claim 1 to comply with the sequence rules and have canceled claim 2 for the same reason. The Examiner's objection to claim 1 is thus negated. Applicants respectfully request the rejection of the claims under U.S.C. §112, second paragraph be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged. If the Examiner believes a telephone conference would aid in the prosecution of this case in any way, please call the undersigned at 415-576-0200.

Respectfully submitted,



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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Amended) A method for reducing a condition associated with fetal alcohol syndrome in a subject who is exposed to alcohol *in utero*, the method comprising administering to the subject an ADNF polypeptide in an amount sufficient to reduce the condition associated with fetal alcohol syndrome, wherein the ADNF polypeptide is a member selected from the group consisting of:

(a) an ADNF I polypeptide having the following amino acid sequence:

$(R^1)_x$-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala-$(R^2)_y$ (SEQ ID NO:3);

(b) an ADNF III polypeptide having the following amino acid sequence:

$(R^3)_w$-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-$(R^4)_z$ (SEQ ID NO:4);

(c) a mixture of the ADNF I polypeptide of part (a) and the ADNF III polypeptide of part (b);

wherein R^1, R^2, R^3, and R^4 are independently selected and are an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected; and

x, y, w, and z are independently selected and are equal to zero or one[;

(d) a full length ADNF I polypeptide which comprises Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala;

(e) a full length ADNF III polypeptide which comprises Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln; and

(f) a mixture of a full length ADNF I polypeptide of part (d) and a full length ADNF III polypeptide of part (e)].

4. (Once amended) The method of claim [3]1, wherein for the ADNF I polypeptide x and y are both zero.

5. (Once amended) The method of claim [3]1, wherein for the ADNF I polypeptide:

x is one;

R^1 is Val-Leu-Gly-Gly-Gly (SEQ ID NO:5); and

y is zero.

6. (Once amended) The method of claim [3]1, wherein for the ADNF I polypeptide:

x is one;

R^1 is Val-Glu-Glu-Gly-Ile-Val-Leu-Gly-Gly-Gly (SEQ ID NO:6); and

y is zero.

7. (Once amended) The method of claim [3]1, wherein for the ADNF III polypeptide w and z are both zero.

8. (Once amended) The method of claim [3]1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Gly-Gly; and

z is zero.

9. (Once amended) The method of claim [3]1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Leu-Gly-Gly;

z is one; and

R^4 is Gln-Ser.

10. (Once amended) The method of claim [3]1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Leu-Gly-Leu-Gly-Gly (SEQ ID NO:7);

z is one; and

R^4 is Gln-Ser.

11. (Once amended) The method of claim [3]1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly (SEQ ID NO:8);

z is one; and

R^4 is Gln-Ser.

13. (Once amended) The method of claim [3]1, wherein x, y, w, and z are all zero.

APPENDIX B

PENDING CLAIMS CURRENTLY UNDER EXAMINATION

1. (Twice amended) A method for reducing a condition associated with fetal alcohol syndrome in a subject who is exposed to alcohol *in utero*, the method comprising administering to the subject an ADNF polypeptide in an amount sufficient to reduce the condition associated with fetal alcohol syndrome, wherein the ADNF polypeptide is a member selected from the group consisting of:

(a) an ADNF I polypeptide having the following amino acid sequence:

$(R^1)_x$-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala-$(R^2)_y$ (SEQ ID NO:3);

(b) an ADNF III polypeptide having the following amino acid sequence:

$(R^3)_w$-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-$(R^4)_z$ (SEQ ID NO:4);

(c) a mixture of the ADNF I polypeptide of part (a) and the ADNF III polypeptide of part (b);

wherein R^1, R^2, R^3, and R^4 are independently selected and are an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected; and

x, y, w, and z are independently selected and are equal to zero or one.

4. (Once amended) The method of claim 1, wherein for the ADNF I polypeptide x and y are both zero.

5. (Once amended) The method of claim 1, wherein for the ADNF I polypeptide:

x is one;

R^1 is Val-Leu-Gly-Gly-Gly (SEQ ID NO:5); and

y is zero.

6. (Once amended) The method of claim 1, wherein for the ADNF I polypeptide:

x is one;

R^1 is Val-Glu-Glu-Gly-Ile-Val-Leu-Gly-Gly-Gly (SEQ ID NO:6); and

y is zero.

7. (Once amended) The method of claim 1, wherein for the ADNF III polypeptide w and z are both zero.

8. (Once amended) The method of claim 1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Gly-Gly; and

z is zero.

9. (Once amended) The method of claim 1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Leu-Gly-Gly;

z is one; and

R^4 is Gln-Ser.

10. (Once amended) The method of claim 1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Leu-Gly-Leu-Gly-Gly (SEQ ID NO:7);

z is one; and

R^4 is Gln-Ser.

11. (Once amended) The method of claim 1, wherein for the ADNF III polypeptide:

w is one;

R^3 is Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly (SEQ ID NO:8);

z is one; and

R^4 is Gln-Ser.

12. (Once amended) The method of claim 1, wherein the ADNF polypeptide is a mixture of ADNF I polypeptide of part (a) and the ADNF III polypeptide of part (b).

13. (Once amended) The method of claim 1, wherein x, y, w, and z are all zero.

15. (As filed) The method of claim 1, wherein the condition is a decreased body weight of the subject.

16. (As filed) The method of claim 1, wherein the condition is a decreased brain weight of the subject.

17. (As filed) The method of claim 1, wherein the condition is a decreased level of VIP mRNA of the subject.

18. (As filed) The method of claim 1, wherein the condition is death of the subject *in utero*.

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On 12/26/01

TOWNSEND and TOWNSEND and CREW LLP

By:

#17
DGJ
4/1/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Douglas E. Brenneman, *et al.*

Application No.: 09/267,511

Filed: March 12, 1999

For: PREVENTION OF FETAL ALCOHOL SYNDROME AND NEURONAL CELL DEATH WITH ADNF POLYPEPTIDES

Examiner: Sharon Turner

Art Unit: 1647

DECLARATION OF DR. DOUGLAS E. BRENNEMAN UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, Douglas E. Brenneman, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I, Dr. Brenneman, am currently Senior Investigator and Chief of the Section on Developmental and Molecular Pharmacology at the National Institute on

Child Health and Development/National Institutes of Health. I received my Ph.D. in Pharmacology and Toxicology from the University of Kansas in 1980. I joined the NIH in 1980 as a post-doctoral fellow. In 1992, I became Chief of the Section on Developmental and Molecular Pharmacology at the National Institute on Child Health and Development/National Institutes of Health. A copy of my curriculum vitae is attached hereto as Exhibit 1.

3. The present invention provides polypeptides that are effective in reducing a condition associated with fetal alcohol syndrome in a subject who is exposed to alcohol *in utero*. The polypeptides are the ADNF I and ADNF III proteins or mixtures thereof that comprise a core active site domain. The conserved core active site of ADNF I comprises the amino acid sequence SALLRSIPA and is known as "ADNF-9" or "SAL." ADNF-9 is the smallest peptide that exhibits the same activity as full-length ADNF I. The conserved core active site of ADNF III comprises the amino acid sequence NAPVSIPQ and is known as "NAP." NAP is the smallest peptide that exhibits the same activity as full-length ADNF III.

4. I am a named inventor on the above-referenced patent application. I have read and am familiar with the contents of this patent application. In addition, I have read the Office Action, dated June 29, 2001, received in the present case. It is my understanding that the Examiner is concerned that the claimed methods are not enabled by the specification. Specifically, the Examiner states that the specification is enabling for a method of inhibiting fetal demise, decreased fetal birth weight, and decreased fetal brain weight in a subject exposed to alcohol *in utero*: the method comprising administering to the subject an ADNF I polypeptide, or an ADNF III polypeptide, or a mixture of both, before the intraperitoneal administration of alcohol. Office action at page 5. However, the Examiner states that the specification is

not enabling for subjects who have already developed fetal alcohol syndrome, prior to treatment with ADNF I or ADNF III polypeptides.

5. This declaration is provided to demonstrate that practice of the claimed methods is fully enabled by the specification. This declaration presents experiments in which pregnant mice were treated with ADNF I or ADNF III polypeptides either before or after administration of alcohol. The effect of the ADNF I or ADNF III treatment on fetal death, fetal brain weight, and fetal body weight was determined. The experiments herein were done under my supervision. The results demonstrate that treatment with ADNF I and ADNF III polypeptides is effective in reducing conditions associated with fetal alcohol syndrome when given before or after administration of alcohol. One of skill in the art can therefore practice the claimed methods using information provided in the specification, together with methodology known to one of skill in the art, with at most, only routine experimentation.

6. The model used is an art-accepted model for fetal alcohol syndrome. For accurate and reproducible administration of alcohol and peptide, a model utilizing intraperitoneal injection was chosen. Webster *et al. Neurobehav. Toxicol.* 2:227-234 (1980) (Exhibit 2). The highest alcohol dose in the model (0.03 ml/kg) was selected to provide the most severe test of efficacy. Using this model, administration of alcohol on gestational day 8 resulted in the highest rate of fetal demises and anomalies. Thus, gestational day 8 was chosen as the optimal and most severe test for the protective activity of the peptides. Previous studies indicated that the intraperitoneal model results in higher blood alcohol concentrations than obtained by an oral route, providing a stringent test to evaluate treatment efficacy.

7. The experiments described below were presented in Spong, *et al.*, *J. Pharm. Ex. Ther.* 297:774-779 (2001) (Exhibit 3). These experiments were conducted

according to methodology taught in the instant application, using the art-accepted model. In one experiment, 0.030 ml/g of body weight of alcohol was administered intraperitoneally to pregnant mice on gestational day 8. Alcohol administration resulted in approximately one third of the fetuses dying. For control mice that did not receive alcohol, less than 5% of fetuses died. The fetal survival rate of mice treated with "NAP" (an ADNF III polypeptide) or with a combination of "ADNF-9" (an ADNF I polypeptide) and NAP polypeptides thirty minutes before alcohol administration was the same as that of the control mice and was significantly higher than the survival rate of fetuses from mice administered alcohol alone. Importantly, treatment with NAP, alone or in combination with ADNF-9, one hour after alcohol administration, also resulted in protective effects against fetal death. The fetal survival rate of pregnant mice treated with NAP, alone or in combination with ADNF-9, one hour after alcohol administration was significantly higher than the fetal survival rate of mice given alcohol alone. However, a three hour post-treatment with NAP and ADNF-9 did not result in a significant change in the number of surviving fetuses in comparison with the alcohol treated group. These results are summarized in Figure 2 of Spong *et al.* These results show that treatment with ADNF III, alone or in combination with ADNF I polypeptide, after alcohol administration will reduce the fetal death rate.

8. The effect of ADNF-9 or NAP polypeptides on alcohol-induced fetal growth abnormalities was determined. Pregnant mice given alcohol had litters with significantly smaller individual fetal brain and body weights compared to control mice that were not given alcohol. Pre-treatment with ADNF-9 and NAP polypeptides prevented the alcohol-associated growth restrictions. Treatment with ADNF-9 and NAP polypeptides, either one or three hours after alcohol administration, prevented the alcohol-induced reduction in brain weight. Fetal brain weights in litters from pregnant mice treated with ADNF-9 and NAP polypeptides, either one or three hours after alcohol administration, were significantly different than fetal brain weight of litters from mice

administered alcohol alone. Post-treatment with ADNF-9 and NAP had little effect on fetal body weight. The results just described are summarized in Figure 3 of Spong *et al.* These results show that treatment with ADNF III and ADNF I polypeptides after alcohol administration will reduce the occurrence of conditions associated with fetal alcohol syndrome, such as reduction in fetal brain weight.

9. In another experiment, pregnant mice were given [^{3}H]NAP, the core active site peptide of ADNF III, by intraperitoneal injection. Fetal tissue samples were collected and analyzed. [^{3}H]NAP was recovered from embryos, demonstrating that after administration to the mother, NAP is transferred to the fetus. Size exclusion chromatography, shown in Figure 4 of Spong *et al.*, was used to analyze the integrity of [^{3}H]NAP found in the embryo. Thirty minutes after administration to the mother, the majority of [^{3}H]NAP recovered from embryos was intact protein. Twenty micrograms of NAP was given to pregnant mice. The estimated concentration of NAP recovered from embryos was 10 nM, within the disclosed therapeutic range of NAP.

10. Using the art-accepted model of intraperitoneal alcohol administration to pregnant mice, ADNF I and ADNF III polypeptides have been shown to reduce conditions associated with fetal alcohol syndrome when given before or after alcohol administration. Specifically, treatment with NAP, alone or in combination with ADNF-9, one hour after alcohol administration, resulted in increased rates of fetal survival, when compared to mice given alcohol alone. Treatment with ADNF-9 and NAP polypeptides together, either one or three hours after alcohol administration, prevented alcohol-induced reduction in brain weight. Using [^{3}H]NAP, the peptide was shown to be transferred from the mother to the fetus and a therapeutic dosage range was confirmed.

12. In view of the foregoing, it is my scientific opinion that one of skill in the art would be able to practice the claimed invention with, at most, routine experimentation. The specification, therefore, fully enables the methods of the invention.

Date: 12/21/01 By: _____
Douglas E. Brenneman, Ph.D.